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14-C-Naphthyl, 14-C-Methyl and 14-C-Carbonyl Carbaryl Distribution in the Pregnant Mouse and Rat

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^{14}C-Naphthyl, ^{14}C-Methyl and ^{14}C-Carbonyl Carbaryl Distribution in the Pregnant Mouse and Rat

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16. ABSTRACT

The distribution of carbaryl labeled with ^{14}C in the ring, methyl or carbonyl groups was determined in pregnant mice and rats. Three identical concurrent experiments were performed using each of the three radiolabeled compounds in each of the species so that the different moieties of carbaryl could be traced through maternal tissues and across the placenta to the fetus for both assay of activity and identification of carbaryl. Following a single dose in the maternal tissues, the concentration of carbaryl ^{14}C-equivalents peaked at 1-2 hours and declined steadily to 24 hours for all three labels in the mice. In rats, the naphthyl label concentration declined steadily for 24 hours but not that of the methyl or carbonyl label. The naphthyl label was the best estimator of carbaryl concentration in both mice and rats. In the fetuses and placentas of mice and rats both carbaryl and metabolites were detected. The fetuses and placentas retained the methyl and carbonyl labeled groups and use of these labels could resul inerroneously high estimates of carbaryl.

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SUMMARY

The distribution of carbaryl labeled with ^{14}C in the ring, methyl or carbonyl groups was determined in pregnant mice and rats. Three identical concurrent experiments were performed using each of the three radiolabeled compounds in each of the species so that the different moieties of carbaryl could be traced through maternal tissues and across the placenta to the fetus for both assay of activity and identification of carbaryl. Following a single dose in the maternal tissues, the concentration of carbaryl ^{14}C-equivalents peaked at 1-2 hours and declined steadily to 24 hours for all three labels in the mice. In rats, the naphthyl label concentration declined steadily for 24 hours but not that of the methyl or carbonyl label. The naphthyl label was the best estimator of carbaryl concentration in both mice and rats. In the fetuses and placentas of mice and rats both carbaryl and metabolites were detected. The fetuses and placentas retained the methyl and carbonyl labeled groups and use of these labels could result in erroneously high estimates of carbaryl.

INTRODUCTION

Carbaryl (1-naphthyl N-methylcarbamate), also known as Sevin®, is a monomethyl carbamate used on hundreds of food, fiber and forage crops as well as on livestock and pets. It is the most commonly used carbamate insecticide and is generally considered to have low acute toxicity with LD-50s in mice and rats of approximately 500 mg/kg. Carbaryl is a small molecule that is uncharged in aqueous solutions which allows movement into cells by diffusion. Carbaryl is also lipophilic with an olive oil/water coefficient of 64.5 (1).

There are reports indicating that carbaryl is teratogenic or fetotoxic in a variety of species (2-7). In some of these reports, the dose levels were high or the route of administration was by injection raising some doubts as to the applicability of these studies to hazard assessment. Other doubts were raised as to the use of the beagle dog because the dog metabolizes carbaryl differently from the rat (8) while the rat metabolizes carbaryl in a more similar fashion to the human being (9,10).

When considering of whether or not carbaryl is teratogenic or fetotoxic, it is also necessary to question whether or not carbaryl reaches the fetus since it is quickly and almost completely metabolized to many products within 24 hours (11). If intact carbaryl does not reach the fetus, then it cannot be a direct teratogenic hazard, although it is conceivable that some metabolites of carbaryl could be teratogenic and thus indirectly carbaryl could be a teratogen. However, if it does reach the fetus, carbaryl might have some teratogenic potential. In those cases, it should be determined whether or not the adverse effects of carbaryl are related to its cholinesterase inhibitory properties since similar results could be expected from any cholinesterase inhibitor.

However, since carbaryl affects many biochemical reactions other than the inhibition of cholinesterase, adverse fetal effects could occur regardless of the state of the cholinesterase enzymes (11).

Since the differences reported in fetal response in the various species may reflect the quantity of carbaryl crossing the placenta and reaching the fetus, this study was undertaken to determine the placental transfer of carbaryl. The use of three radiolabels of carbaryl, as described by Dorough, were used (12). They are shown in Figure 1. Carbaryl was labeled with ^{14}C in the ring structure to produce ^{14}C naphthyl carbaryl; in the N-methyl group to produce ^{14}C methyl carbaryl; and in the carbonyl group to produce ^{14}C carbonyl carbaryl. Three identical concurrent experiments in both mice and rats were performed using each of the radiolabeled compounds so that the different moieties of carbaryl could be traced through the maternal tissues and across the placenta to the fetus for both assay of activity and identification of carbaryl. The mouse and rat were chosen for these studies since in some mouse strains, carbaryl has been shown to produce fetal malformations (5) while reproduction studies in rats have demonstrated adverse effects of carbaryl on reproduction at high dose levels (6,7).

MATERIALS AND METHODS

Radiolabeled carbaryl (99+% purity) was obtained from Amersham Corp., Ill. The ^{14}C label was located in one of three positions:

1. (1-naphthyl-^{14}C)-N-methyl carbamate, 5.8 mCi/mmol.
2. 1-naphthyl-N-(methyl-^{14}C) carbamate, 6.3 mCi/mmol.
3. (carbonyl-^{14}C)-1-naphthyl-N-methyl carbamate, 59.9 mCi/mmol.

Nonradiolabeled 1-naphthyl-N-methyl carbamate (carbaryl) of 99% purity was obtained from City Chemical Co., Inc., N.Y.

1. Assay of Carbaryl

Carbaryl was assayed by two methods: first, direct quantification of radioactivity in tissue samples; second, by thin layer chromatography with identification of the spots chromagenically and scraping the spots and quantifying the radioactivity. Duplicate thin layer plates were exposed to X-ray film.

a. Radioactivity Assay

^{14}C-Carbaryl was assayed in tissue samples or tissue homogenates by combustion in a Packard Tri-Carb Sample Oxidizer, Model B-306 using Carbo-Sorb to capture the carbon dioxide. A few tissues were also digested in Soluene-100. Radioactivity was determined using Permafluor V as the scintillation fluid and a Mark III Scintillation Counter, Searle Corp., Ill. Soluene-100 and Permafluor V are products and registered trade marks of Packard Instruments Co., Inc., Ill.

b. Thin Layer Chromatographic Assay

Aliquots of the homogenates were extracted and then assayed by thin layer chromatography using Uniplate, Silica Gel G, 250 μm thick, Analtech,

Inc., DL. Acetone, methyl alcohol and methylene chloride were pesticide grade solvents from Burdick and Jackson, Inc., MI and the benzene and diethylene glycol were reagent grade from Fisher Scientific Co., N.C. The extraction and chromatographic procedures were adapted from the official 1975 AOAC method (13), as modified by Sherma et al. (14). The adaptations were use of small sample sizes with volumes of homogenates ranging from 1 to 5 ml, and extraction with the least amount of methylene chloride possible. After addition of the ammonium sulfate, methylene chloride was added in sufficient quantity to just separate the gel, and the volume was recorded. This usually required a volume of methylene chloride of 0.5 to 1.0 ml/ml of homogenate. The methylene chloride extracts were spotted directly on the thin layer plates; 50 µl of the extracts of rat tissue and 100 µl of the extracts of mouse tissue were utilized. Each plate had a ^{14}C-carbaryl standard of approximately 80-100 dpm with 4 µg of nonradioactive carbaryl. Also, 4 µg of nonradioactive alpha-naphthol was added to the carbaryl standard. The chromatographic plates were developed in an acetone-benzene solution (1+4). After drying for approximately 10 minutes, the plates were sprayed with the chromagen (saturated diethylene glycol-alcohol solution (1+9) with p-nitrobenzene diazonium fluroborate) which produced an orange/pink color for alpha-naphthol. The plates were then sprayed with 1.0 N KOH in methanol and sprayed with the chromagen which produced a blue color for carbaryl.

Two identical plates were prepared for all tissues. One plate was used for color development of the spots. The spots which appeared were subsequently scraped and assayed for radioactivity. When carbaryl or alpha-naphthol were not evident after spraying, areas corresponding to

the Rfs of these compounds were scraped. Permafluor V was added to the scrapings, and the level of radioactivity was determined in the scintillation counter. The other plate was exposed to X-ray film for two months to detect any radioactive spots.

c. Extraction Efficiency from Chromatographic Plates

An extraction efficiency was determined for the recovery of carbaryl from the chromatographic plates. Standard solutions of each of the ^{14}-C-carbaryls in methylene chloride were spotted on the plates, developed, and four areas scraped as follows: a. area from the bottom of the plate to a point one inch above the origin; b. area starting at a point one inch above the origin to one inch below the carbaryl Rf; c. area from a point one inch below the carbaryl Rf to the midpoint between the carbaryl and the alpha-naphthol Rf; d. area from the midpoint between the carbaryl and alpha-naphthol Rfs to the solvent front. The recoveries were as follows: areas a, b and d had recoveries of 1% or less of the applied radioactive carbaryls; area c had 88%±2 and 87%±1 recoveries of the radiolabeled carbaryls at levels of approximately 500 dpm and 100 dpm respectively. Each recovery is based on 6-7 replicates.

d. Extraction Efficiency from Tissues

An extraction efficiency was determined for tissues. Two tissue types were used, the high lipid tissues, i.e. adipose tissue and brain; and those with low lipid content, i.e. muscle, liver, heart and kidney. Homogenates in water of these tissues were fortified with ^{14}C-carbaryl at radioactivity levels of 860 dpm, 430 dpm or 80 dpm. There were 5-7 replicates at each fortification level. The recoveries were as follows:

	$\bar{X}$ PERCENT RECOVERY (±S.E.M.) FORTIFICATION LEVEL		
TISSUE	860 dpm	430 dpm	80 dpm
low lipid	76.3 ± 1.3	68.9 ± 0.5	70.0 ± 3.2
high lipid	64.0 ± 2.7	60.2 ± 3.5	55.3 ± 3.2

These data are presented as an estimate of the extraction efficiency of carbaryl from homogenates used for the thin layer chromatography. Tissues and homogenates which were used to determine carbaryl concentration were combusted directly without extraction.

Both radioactive and nonradioactive standards of carbaryl were run on thin layer to determine the minimum detectable concentration. For the chromagenic assay with a 100 µl application, 100 ng of carbaryl were barely detectable and 400 ng were readily detectable. Also, 400 ng of alpha-naphthol were readily detectable. For the X-ray film assay, 60 dpm of carbaryl were barely detectable after two months exposure.

At the end of the experiment, the radiolabeled carbaryl was assayed for purity by thin layer chromatography. There was no evidence of breakdown and the purity did not differ from that reported above.

2. Animal Studies

a. Study One

Adult female CD-1 mice and CD rats were obtained from Charles River Laboratories, Inc., Wilmington, MA. The mice were bred in the laboratory, but the rats were purchased with known gestation dates. Detection of a

vaginal plug or sperm indicated day zero of gestation. Mice were treated concurrently by intragastric intubation with either ^{14}C-naphthyl-carbaryl, ^{14}C-methyl-carbaryl or ^{14}C-carbonyl-carbaryl on day 13 of gestation. The mice and rats received 100 mg/kg of carbaryl solution in corn oil at a volume of 0.4 ml/mouse and 1.5 ml/rat. The gestational day 12 body weights of the mouse and gestational day 13 body weights of the rats were used to calculate the dosing solutions. Each radiolabeled carbaryl solution contained 5 µCi/mouse or rat. The nonradioactive carbaryl was dissolved in acetone before adding corn oil so that the dosing solution was 1:9 acetone:corn oil. The radiolabeled carbaryls were in solution in benzene of which 500 µl or less were added to approximately 5 ml of the dosing solutions.

The mice were sacrificed at 1, 3, 5, 7 or 24 hours after treatment by decapitation. There were 2 mice per treatment period. Tissues were removed, weighed and placed in scintillation vials with Soluene 100 or into a cone for combustion. Rats were treated similarly with the radiolabeled carbaryls on day 14 of gestation and sacrificed at 1, 2, 4, 8, 12 or 24 hours after treatment. There were two rats per time period. All tissue samples were taken for combustion. All mouse and rat tissues were assayed in duplicate except the mouse fetuses and placentas which were assayed in 3 or more replicates. The fetuses were analyzed either individually or pooled two or three to a sample and each analysis was considered an assay. The respective placentas were treated the same as the fetuses. Pooled urine and pooled feces samples were collected from the mice and rats which were sacrificed at 12 and 24 hours.

b. Study Two

CD-1 mice and CD rats were purchased with known gestation dates from Charles River Laboratories, Inc., Wilmington, MA. The mice were treated intragastrically with 100 mg/kg of the radiolabeled carbaryl dosing solutions on day 13 of gestation as in study one but sacrificed at 1 or 4 hours after treatment. There were six mice per time period. Samples of tissues were taken and combusted to determine radioactivity. An additional sample of tissue was homogenized and aliquots used for analysis by thin layer chromatography or combustion to determine radioactivity. The rats were treated intragastrically with 80 mg/kg of the ^{14}C-naphthyl-carbaryl or ^{14}C-methyl-carbaryl radiolabeled carbaryl dosing solutions on day 14 of gestation and sacrificed at 1 or 4 hours after treatment. There were 6 rats per time period. The tissues were homogenized and aliquots taken for determination of radioactivity and for analysis by thin layer chromatography. Tissues of mice and rats were assayed in 2-4 replicates per tissue.

The fetuses were pooled 2/sample, and the respective placentas were also pooled 2/sample. Two to four samples were assayed per animal.

3. Presentation and Statistics

The data in this report are from pregnant animals only. The data from the few nonpregnant animals indicated no observable difference between the pregnant and nonpregnant animals, but the size of any one nonpregnant treatment group was too small for proper evaluation.

Data are presented as µg carbaryl equivalents/gm wet tissue weight calculated using standard solutions of the three radiolabeled compounds carried throughout the entire procedure and internal standards to determine counting efficiency.

The litter was considered the unit for all calculations. Thus, means were taken for each litter and then averaged across litters. All data are presented as the means and SEMs. The data were analyzed statistically using ANOVA and Duncan's Multiple Range Test (15).

RESULTS

I. Study One

In study one, the distribution of the labeled carbaryls was determined in mice at 1, 3, 5, 7, and 24 hrs after treatment, and in rats at 1, 2, 4, 8, 12 and 24 hrs after treatment. Although there were two test animals per time period, only data from animals which were pregnant are included in the graphs. The bars in the graphs without the standard error indicator marks represent one animal.

A. CD-1 Mouse Study

The distribution of ^{14}C-naphthyl, ^{14}C-methyl and ^{14}C-carbonyl carbaryl in tissues of pregnant CD-1 mice of study one is presented in Figures 2 and 3. The scale for the concentrations of the radiolabeled carbaryls is the same for all tissues except liver and kidney. For liver and kidney, the scales were adjusted to accomodate the high concentrations. In order to make direct comparisons among the three labels, it is essential that the doses be the same. Since a number of the tissues had the same concentration of radiolabels at one hour, it was assumed that equivalent doses were achieved.

The peak concentration of the naphthyl label was at one hour for all tissues except skin for which the peak concentration was at three hours. The decline in the naphthyl label was fairly rapid in all tissues

with low but measurable levels by 24 hours. The concentration of the methyl label appeared to be equivalent to the concentration of the naphthyl label at one hour in liver, fat, kidney, and skin. In contrast to those tissues, the concentration of the methyl label exceeded that of the naphthyl up to four-fold in the brain, heart, eye, and muscle. The concentration of the methyl label was less than the concentration of the naphthyl label only in the blood at one hour. By three hours, the concentration of the methyl label had increased in all tissues except fat. This was in contrast to the concentration of the naphthyl label which showed a decrease. The concentration of the methyl label remained high in most tissues through 7 hours but declined by 24 hours at which time it was still readily detectable in all tissues.

The distribution of the carbonyl label showed a slightly different pattern of distribution compared to the naphthyl or methyl label. At one hour, the concentration of the carbonyl label was similar to that of the naphthyl label in the liver, brain, fat, heart, eye, lung and muscle. However, the concentration in the blood, kidney, and skin was lower than that seen with the naphthyl label. In general, the concentration of the carbonyl label, like the naphthyl label, was less than the concentration of the methyl label in most tissues for all time periods. The decline in the concentration of the carbonyl label with time was less than that of the naphthyl or methyl label. The concentration remained fairly constant in the brain, heart, blood, eye, skin and muscle although the concentration in the other tissues showed a definite decrease by 24 hours.

In summary, the pattern of distribution of carbaryl differed somewhat among the three radiolabels. Since all three of the radiolabels represent

equal doses of carbaryl, the different patterns reflect the distribution of both the parent compound and the metabolites or fragments of the carbaryl molecule. Because the amount of intact carbaryl can be no greater than the least concentration of any label at any time period, the concentration of carbaryl cannot exceed the concentration of the naphthyl label which was the least for almost all tissues and time periods.

In Figures 2 and 3, it is obvious that the methyl label is prominent in all tissues at all time periods. Since the methyl group is labile, it is suggested that the methyl group was readily cleaved from the carbaryl molecule and incorporated into methyl pools or directly into tissue components.

The concentration of the carbonyl label was less than that of the naphthyl label in some tissues, and this would indicate that the concentration of carbaryl was no greater than the low level of the carbonyl label in those tissues. Of interest with the carbonyl label is the concentration in the eye, brain, and heart which maintain a fairly steady concentration over time. This would suggest that the carbonyl moiety might be retained by these tissues since the concentration of the naphthyl label decreased with time.

B. CD Rat Study

The patterns of distribution of the three radiolabeled carbaryls in tissues of pregnant rats of study one are presented in Figures 4 and 5. The concentrations of each of the labels at one or two hours were equivalent for all tissues suggesting that equivalent dosages were achieved. The concentration of the naphthyl label generally decreased with time to

low, but detectable, levels at 24 hours. In all tissues there appeared to be a slight increase in concentration at 8 hours followed by a decrease in concentration at 12 hours. It is not known whether this is an artefact or perhaps redistribution of the carbaryl. The concentrations of the methyl and naphthyl labels at one and four hours were generally the same in all tissues. However, in contrast to the naphthyl label, the concentration of the methyl label did not decline in all tissues by 24 hours. The eye, brain, kidney, liver, heart and muscle showed an increase in concentration of the methyl label at 24 hours following an initial decline while the fat and skin had steadily decreasing concentrations over the 24 hours. In marked contrast, the blood and lung had increased concentrations of the methyl label at 24 hours compared to one hour. It is not known whether the methyl label was being retained by these two tissues or was in the process of being excreted or transported by the blood as metabolites.

A modest decrease in concentration of the carbonyl label in the liver and kidney and a definite decrease in this label in the fat was noted over the 24 hours. Very little decrease in concentration was found in the eye, brain, blood, lung, heart, skin and muscle. Since the concentration of the naphthyl label decreased in most of these tissues, it appears that the carbonyl moiety was retained. Thus, the concentration of carbaryl would be no greater than the concentration indicated by the naphthyl label which had the lowest concentration of the three radiolabels.

C. Fetal and Placental Concentrations

The concentrations of the radiolabeled carbaryls in the fetuses and placentas of the mice and rats of study one are presented in Table 1. The mouse fetuses were analyzed by either solubilizing in Soluene-100 or

combusting and capturing the CO_2. The number of samples processed by solubilizing in Soluene-100 was too small for a proper statistical analysis and have not been included in the table. However, upon inspection, the data from the two procedures did not appear to differ. Subsequent studies used the combustion technique. In the mouse study, most of the fetuses and placentas within a litter were studied to determine the variability within a litter for this type of assay. With the limited amount of data available, it appeared that the range of the variability within litter was low and was lower than the range of the variability between litters. Therefore, in the rat study and in study two for both the mouse and rat, 2 fetuses or 2 placentas were combined per assay, and two assays were performed per litter.

1. CD-1 Mouse

In the mouse fetus, the concentration of the naphthyl label was low and declined during the 24 hours. The concentration of the methyl label greatly exceeded that of the naphthyl label at all times. Although the methyl concentration increased during the first 7 hours following treatment, by 24 hours it had decreased and was lower than the one-hour level. The carbonyl label concentration was similar to the naphthyl label concentration at one hour, but increased in concentration through 7 hours. By 24 hours the concentration of the carbonyl label had decreased to a level similar to, but still statistically different from the level of the methyl label.

Since the concentration of carbaryl cannot exceed the concentration of the naphthyl label, the lowest value of the three labels, the high concentrations of the methyl and carbonyl labels reflect the possible incorporation of the methyl and carbonyl moieties into the fetus. The

growth of the fetus in 24 hours is sufficient to produce a dilution of the ^{14}C which would contribute to the decline in concentration at 24 hours.

The concentration of the naphthyl label in the placentas at one hour was about 3 times higher than the concentration in the fetus. The concentration of the label was steady over the first seven hours, but by 24 hours there was a decline. The concentration of the methyl label in the placentas was only twice that seen in the fetuses and did not increase during the seven hours in which the fetal concentrations doubled. By 24 hours, the concentration of the methyl label in the placentas had decreased by half from the seven hour values but still remained quite high. The concentration of the carbonyl label in the placenta was about twice as high as that seen in the fetus. The concentration remained constant for five hours; showed a slight increase at 7 hours and a decrease by 24 hours to a level below its one-hour concentration. The pattern of the carbonyl label in the placenta was very similar to that of the fetus. Again, as in the case of the mothers, the concentration of carbaryl is best estimated by the naphthyl label at most time periods in both the fetuses and placentas.

2. CD Rat

In the rat at one hour, the concentrations of the three radiolabels were similar to each other both in the fetuses and placentas, but by 12 hours the concentration of the methyl label had risen and was significantly higher than the concentration of either the naphthyl or carbonyl labels. The concentration of the naphthyl label in the fetus decreased steadily from one hour to 24 hours. The concentration of the naphthyl label in the placenta was twice that seen in the fetus at one hour, but decreased

steadily over the 24 hours to a concentration similar to that seen in the fetus at 24 hours.

The concentration of the methyl label in the fetus had a very irregular pattern over the 24-hour period. For the first four hours, the concentration was generally constant, but the large standard errors indicate large variances among the fetal values. At 8 hours, there was a decrease in the concentration which was followed by a marked increase at 12 and 24 hours so that the 24-hour concentration of the methyl label was twice that of the one-hour concentration. It is not known if this irregular pattern merely reflects the variation among animals or the possibility of more complex dynamics such as the elimination of carbaryl confounded with the incorporation of the methyl moiety into the fetus. The concentration of the methyl label was slightly higher in the placenta compared to the fetus, but the large standard error reflects great variability. The concentration was constant for the first 8 hours with an increase by 12 and 24 hours as was seen in the fetuses. Also, the doubling of the concentration at 24 hours compared to one hour was the same pattern noted in the fetuses. It is not known whether the placenta could incorporate the methyl label since the placenta should have reached its final size by this stage, or whether the increase in the methyl label merely reflects the methyl pool or the transport to and from the fetus.

The concentration of the carbonyl label in the fetus was constant with very small variances throughout the 24 hours. The concentrations at one and 24 hours were not significantly different. The concentration of the carbonyl label in the placenta was slightly higher than that seen in the fetus and the variance was greater especially at 2 and 8 hours.

Otherwise, the concentration remained constant over time. Apparently, the carbonyl moiety was retained by both the placenta and fetus. Since the naphthyl label concentration was the least of the three at all time periods, the concentration of carbaryl could not exceed that of the naphthyl label. It would also indicate that by 24 hours, there was very little carbaryl in either the fetuses or placentas. As with the other results presented so far, the naphthyl label concentration was probably the most accurate indicator of carbaryl concentration.

D. Chromatographic Assay

To determine whether carbaryl was actually present in the tissues discussed in the previous sections, thin layer chromatography was performed. The results are presented in Table 2. The "C" in the table represents carbaryl. The numbers indicate radioactive metabolites and the number is the metabolite Rf expressed as a percent of the carbaryl Rf as a means of normalizing the data. A metabolite is only listed once although it may have been detected in more than one rat. The percent Rf indicative of alpha-naphthol is in the range of 110-118 with a mean of 114.

In general, parent carbaryl was detected in all tissues including the fetuses and placentas. Very few metabolites were detected with the methyl label. Although some metabolites were detected with the naphthyl label, most of the metabolites were labeled with the carbonyl group. A possible explanation is that the carbonyl labeled metabolites were the least volatile and were more readily detected. As anticipated, the liver and kidney had the majority of all types of metabolites although a number were detected in the heart, lung, and brain. The skin, fat and blood had the least number of metabolites.

E. Excretion of Radiolabels

In both mice and rats, the naphthyl label concentration in the tissues decreased to very low values in 24 hours. This was partly due to excretion (Figure 6). The concentration of the three radiolabels in the pooled urine and pooled feces of the mice did not differ greatly at 24 hours. In contrast, in the rat, there was a marked difference in the excretion pattern of the three labels. The naphthyl label was readily excreted mainly in the urine. The quantity of methyl and carbonyl labels excreted in the urine and feces was similar and about a half to two-thirds less than the quantity of the naphthyl label. The greater quantity excreted by the rat reflects the greater quantity received in dosing since both the mouse and rat received 100 mg/kg of carbaryl. When the data are presented as percent of dose excreted, it can readily be seen that the mouse excreted about 60-80% of the carbaryl within 24 hours (Figure 7). Although it appeared that the methyl label was retained for the first 7 hours by some of the tissues of the mouse, the excretion data suggest that the clearance of the three labels was similar by 24 hours. Since less than 100% was recovered, some radiolabel may have been retained by some tissues or excreted as CO_2.

The recovery of the excreted radiolabeled carbaryls from the rat differed greatly from that of the mouse (Figures 6 and 7). While 80% of the naphthyl label was excreted in 24 hours which was similar to the mouse data, only 25 and 40% of the methyl and carbonyl labels, respectively, were excreted in 24 hours. This generally reflects the pattern of radioactivity in the tissues of the rat. Since the recovery of the naphthyl label indicates that 80% of the carbaryl was metabolized/excreted in 24 hours, the same would be expected for carbaryl whether it

was labeled in the methyl or carbonyl position. Thus, it can be assumed that the methyl and carbonyl label were not excreted but retained in the body carbon pools or excreted as CO_2.

II. Study Two

In study two, both the mice and rats were studied at one and four hours after treatment. There were six animals per treatment group, but only data from the pregnant animals are presented. The mice were treated with 100 mg/kg carbaryl, the same dose level as in study one, while the rat dose was decreased from the 100 mg/kg dose used in study one to 80 mg/kg because of observed pharmacologic effects such as vasodilation as noted in the tails and ears as well as generalized depression.

A. CD-1 Mouse

The data from the CD-1 mouse are presented in Table 3. The mouse tissue was studied as either a whole tissue sample or as an homogenate, both of which were combusted. A comparison was made in order to assure that the homogenization procedure and subsequent aliquots were representative of the tissues and that no contamination was carried through the procedure. In general, there were no detectable differences between homogenate and whole tissue means. The few cases of significant differences did not appear to be indicative of any patterns that would suggest consistent variations between whole tissue and homogenate values.

The concentration of the naphthyl label was highest in the liver and kidney, and the concentrations at one hour did not differ statistically from the concentrations at 4 hours. The lung and skin, which had naphthyl label concentrations of about half that seen in the liver and kidney, showed a definite decline in label concentration by 4 hours with the

lung sample label concentration declining significantly. The fat, muscle, uterus and blood, had equivalent but low concentrations at 1 and 4 hours. The brain and eye had very low label concentrations which were comparable at one and four hours. In general, the naphthyl label concentration showed no decline or a slight decline in concentration in most tissues by four hours although the declines were not statistically significant.

In contrast to the naphthyl label, the methyl label showed an increase in concentration in many tissues by four hours. The concentration of the methyl label at one hour was less than that of the naphthyl label but increased by four hours to values similar to those of the naphthyl label. Low concentrations of the methyl label at one hour followed by statistically significant increases in concentrations at four hours were seen in kidney, brain, uterus and blood and possibly liver and lung. The concentration in the eye, muscle and uterus also had increased by four hours, but this increase was not statistically significant. The concentration in fat and liver remained constant while that in skin decreased.

The carbonyl label concentration was significantly increased in many tissues by four hours compared to the one hour concentration. For a number of tissues, the one-hour carbonyl concentrations were much lower than those of either the naphthyl or methyl label while the four-hour concentrations were similar or greater. It might be considered that the dose or initial uptake and distribution of the methyl and carbonyl labels were less than those of the naphthyl label except that the skin, fat, brain and eye had greater concentrations of the methyl label than those of the naphthyl and carbonyl labels. Although the

concentration of the carbonyl label was less than that of the naphthyl label in the liver and kidney at one hour, it was equivalent by four hours. Of note was the marked increase in the concentration of the carbonyl label in the lung at four hours which differs from the decrease seen in the concentration of the naphthyl label. This suggests that the carbonyl moiety may be the source of the CO_2 metabolite. In contrast to the increase in carbonyl label concentration in the lung and skin, the naphthyl label concentration decreased in these tissues while the methyl label concentration was fairly constant. In general, the methyl and carbonyl labels increased in concentration in many but not all tissues while the naphthyl label decreased in concentration.

B. CD Rat

In this rat study, only the naphthyl and methyl labels were used and only samples of homogenates were assayed since the data with the whole tissue and homogenate samples of the mouse differed very little. The concentrations of the naphthyl and methyl labels in the rat tissues are presented in Table 4.

The concentration of the naphthyl label was not significantly different at one and four hours for the liver, kidney, lung, muscle, heart, eye, or blood while the concentration was significantly decreased in the skin by four hours. The concentration of the methyl label was also constant at one and four hours for the liver, kidney, lung, muscle, brain, heart and eye, while there was a significant decrease in label concentration in fat, and an increase in blood and serum. The tissue concentrations of the naphthyl and methyl labels were fairly constant over the four hours and the two labels were equivalent in concentration in the eye and fat and differed in the other tissues. The exceptions

were the organs of excretion, i.e. liver, kidney, lung, blood and serum. The slight but consistent decrease in the concentration of the naphthyl label with time indicates that the concentration of carbaryl decreases similarly with time. The slight fluctuations in the concentration of the methyl label probably reflects the concentration of the parent molecule carbaryl and metabolites as well as radiolabeled methyl groups in the methyl pool.

C. Fetal and Placental Concentrations

1. CD-1 Mouse

The values for the concentrations of the three labels in the fetuses and placentas are presented in Table 5. At one hour, the concentration of the naphthyl label in the fetus was similar to but less than that of the methyl label while both the concentrations were greater than that of the carbonyl label. None were statistically different. By four hours, the concentration of the naphthyl label was increased over the one-hour concentration in both the whole samples and the homogenized samples but the increase was not statistically significant. In both types of samples, the increase was less than two-fold. In contrast, the concentration of the methyl label in the fetus was increased almost five-fold by four hours. In sharper contrast, the increase in the carbonyl label concentration in the fetus was 20-fold by four hours. The final concentrations of the methyl and carbonyl labels were in the same range. The initial values at one hour accounted for the difference in magnitude of the increase.

The distribution of radiolabels in the placentas of these fetuses reflected the same general pattern but the magnitude of the values differed. At one hour in the placentas, the concentration of the naphthyl

label was greater than that of the methyl label and the carbonyl label. There were no significant differences in the concentrations of the naphthyl label at one hour compared to the concentrations at four hours. Both the one and four hour values of the naphthyl label concentration in the placentas were 2 to 3 times greater than their respective fetal values.

At one hour the methyl label concentration was slightly greater in the placenta than in the fetus. By four hours the methyl label had significantly increased by approximately five-fold which is similar to the magnitude of the increase seen in the label concentration in the fetuses but the resulting concentrations were much higher. At one hour, the concentration of the methyl label was less than that of the naphthyl label but by four hours, it was two to three times as great. This increase was comparable to the concentration increase seen in the fetus.

The concentration of the carbonyl label in the placentas was the lowest of the three labels at one hour. There was a statistically significant increase in the concentrations at four hours but the increase was only about 10-fold compared to the 20-fold increase seen in the fetuses.

In general, at all times and with all labels, the concentrations in the placentas were greater than those seen in the fetuses. At one hour, for both fetus and placenta, the label with the lowest concentrations was carbonyl. This would suggest that the concentration of carbaryl could not exceed the concentration determined for the carbonyl label. However, at four hours, the concentration of the naphthyl label was the lowest of the three labels and thus, the concentration of carbaryl could not exceed the concentration determined for the naphthyl label. The

marked increases in the methyl and carbonyl labels in both the fetuses and placentas suggest that fragments of the carbaryl molecule with the methyl and carbonyl labels are being incorporated into the tissues which would indicate erroneously high values for carbaryl if they were assumed to be carbaryl.

2. CD Rat

The values for the radiolabel concentrations in the fetuses and placentas are presented in Table 5. Only the naphthyl and methyl labels were studied. Although the rat received a slightly lower dose (80 mg/kg) than the mouse (100 mg/kg), the one-hour fetal concentrations of both labels are approximately twice those of the mouse.

The concentration of the naphthyl label in the fetus showed a slight nonsignificant decrease by four hours. While the methyl label showed a slight non-significant increase by four hours. Thus, there were no significant changes in either the naphthyl or methyl label concentrations in the rat fetuses by four hours. However, the concentration of the methyl label was significantly greater than that of the naphthyl label. Based on these two labels, the concentration of carbaryl could be no greater than that of the naphthyl label. The pattern of the two labels in the placentas was the same as that in the fetuses. Again, the concentration of carbaryl can be no greater than that of the naphthyl label.

D. Chromatographic Assay

The homogenates of the rat tissue were assayed by thin layer chromatography to determine the presence of carbaryl. Carbaryl was judged to be present if there were either a blue spot at the Rf corresponding to the carbaryl standard or a radioactive spot corresponding to the Rf of

the radioactive carbaryl standard, or both. The numbers in Table 7 indicate the number of rats in which carbaryl or a metabolite was identified. Of the 6 rats treated with the naphthyl label and analyzed at one hour, only one rat had carbaryl in the liver while 3 rats had carbaryl in the kidney and 5 had carbaryl in the lungs. Two fetuses and two placentas had identifiable carbaryl. The few metabolites detected were mainly in the 110-130 range. Alpha-naphthol was found at 115±5% of the carbaryl Rf. At four hours, fewer rats had identifiable carbaryl and more had metabolites. It appears that a large portion of the carbaryl is metabolized within four hours. All the lung samples had metabolites by four hours. Most of the metabolites chromatographed with Rfs similar to alpha-naphthol, but since standards were not available for other metabolites, it can only be suggested that the metabolites in the lung were alpha-naphthol.

Fewer of the rats receiving the methyl label had demonstrable carbaryl in the tissues at one hour compared to those receiving the naphthyl label. There was a slight increase in the number of types of metabolites with the appearance of metabolites in the 52-90 percent range. At four hours, the number of methyl labeled treated rats with demonstrable carbaryl and metabolites was fewer than the number of naphthyl label treated rats. With the methyl label especially and all labels in general, areas of the chromatograph which were scraped, and had measurable radioactivity by liquid scintillation counting did not necessarily have detectable radioactive spots on the X-ray film indicating the high volatility of some of the compounds.

The assay of the mouse tissue homogenates for the presence of carbaryl produced questionable plates due to the very high volatility of

the metabolites and the instability of both the carbaryl and alpha-naphthol colored spots. At low concentrations, as in the mouse study, the spots were visible for a few hours at the most. As the concentration of carbaryl or alpha-naphthol increased, the permanency of the spot increased. Concentrated spots such as the standards were permanent for many weeks, but did eventually fade. There were some doubts as to the reliability of the thin layer plates in the mouse study and thus the data are not presented for study two.

DISCUSSION

The major pharmacologic actions of carbaryl are related to the depression of acetylcholinesterase activity in the blood, brain, nerve and liver. Vandekar et al. (16) have demonstrated a transient but pronounced anaesthetic effect of carbaryl when administered IV or IP at very high dose levels. No such effect was observed following oral administration of carbaryl. From the Russian literature, it has been reported that carbaryl also alters many other enzymes, as well as increases in RNA, DNA, and increases in blood and tissue glycogen. It can alter many of the blood components such as erythrocytes, leukocytes, hemoglobin and serotonin. Kuhr and Dorough have discussed many of these reports in their excellent review (11).

In addition, carbaryl has been shown to have some teratogenic actions and some adverse actions on reproduction. In a teratology study by Robens (2), carbaryl did produce malformations in the guinea pig with single doses of 300 mg/kg which produced 13% maternal mortality. Vertebral malformations were found in almost all of the fetuses exposed on days 12-16 of gestation. Other bone defects were noted as well as agenesis

of the kidneys and genital organs. In the same study, no adverse effects were noted in the fetuses of hamsters and rabbits at dose levels that were not maternally lethal.

In a beagle dog teratology study (3,4), carbaryl administered in the diet at doses up to 50 mg/kg during gestation produced an increased number of difficult births accompanied by the presence of a green-black, foul-smelling discharge from the vagina possibly resulting from placental separation. The incidence of maternal mortality was difficult to judge since parturition was terminated by euthanasia in cases of dystocia. There was an increase in the number of litters with all dead pups. Of the carbaryl treated dogs, 21% produced pups showing fetotoxicity. Other pups were abnormal with anomalies such as: failure of ventral wall to close, acaudate pups, missing coccygeal vertebrae, scoliosis, agenesis of external genitalia, imperforate anus, visceral agenesis to varying degrees and small liver. The postnatal weight gain of the neonates was decreased.

In the Bionetics study with C57BL/6 mice, carbaryl was administered subcutaneously in DMSO at 100 mg/kg from day 6-14 of gestation and reported to produce an increase in hydrocephaly and skeletal malformations. They also studied the AKR strain of mouse at a dose of 464 mg/kg administered subcutaneously in DMSO from day 6-14 of gestation which did not show any teratogenic effects (5).

Carbaryl administered in the diet to rats during gestation or during both gestation and lactation in doses up to 500 mg/kg did not produce any maternal lethality (6). Rats sacrificed during gestation for fetal examination showed that all of the fetuses had normal skeletons; visceral examinations were not done. However, in rats allowed to deliver,

2/3 of the pups died within four days of birth. No adverse neonatal effects were reported at lower dose levels.

Two three-generation reproduction studies with rats have been done. In the study reported by Weil et al. (6), rats were maintained on diets containing carbaryl at dose levels up to 10 mg/kg from weaning throughout the study and no effects were seen on reproduction. Additional rats were treated with either 100 mg/kg by intubation or 200 mg/kg by diet, 5 days/week starting at weaning and continuing throughout the study. Although the 100 mg/kg dose administered by intubation resulted in maternal mortality and reduced fertility, no adverse effects were noted with the 200 mg/kg dose in the diet. Reduction in growth at 100 mg/kg given by intubation was equivalent to that of 200 mg/kg administered in the diet.

In the three-generation reproduction study by Collins et al. (7), carbaryl was administered in the diet at dose levels from 2,000 ppm to 10,000 ppm. The dose of 2,000 ppm in the diet is considered to be equivalent to 100 mg/kg. Fertility was markedly impaired at the highest dose level of 10,000 ppm. At all dose levels, weanling body weights were decreased. At the 2 higher dose levels, litter size, progeny surviving to 4 days of age, and weaning index were all reduced. In general, a decrease in reproductive fitness was evident at all dose levels. From these studies, it can be seen that carbaryl is capable of adversely affecting reproduction and fetal development, albeit not in all species and usually at high dose levels. Because of these adverse effects, some studies concerned with placental transfer and fetal deposition were reported.

Declume and Benard administered ^{14}C-carbaryl, labeled in the N-methyl group, to pregnant mice and rats near the end of gestation for whole body autoradiography. In both rat and mouse fetuses, ^{14}C-activity was seen 5 hours after oral dosing with prominent deposition in the fetal eye, liver and central nervous system (17-18). The radioactivity in maternal organs was assayed and declined over the 96 hour study in the rat, and over the 8 day study in the mouse while the radioactivity in the mouse fetuses increased over those time periods (19,20).

A limited study on rats using ring labeled or carbonyl labeled ^{14}C-carbaryl showed the concentration of the ring label decreased and the concentration of the carbonyl label increased in rat fetal tissues for 8 hours following a single intraperitoneal injection. Preliminary data of the extraction and assay of fetal tissue at 30 minutes identified some carbaryl as being present with a large percentage of the extract being naphthol (21).

There are numerous studies which show that carbaryl is metabolized to a variety of products by hydrolysis, oxidation, and dealkylation resulting in polar metabolites which are readily excreted as conjugates and as CO_2 (11). A number of the metabolites of carbaryl are further metabolized producing an extensive number of breakdown products, some of which are volatile (12). Most of these reports are concerned with metabolites and the means by which they are formed rather than the concentration of carbaryl present in the tissues. In considering the adverse actions which carbaryl has on the developing fetuses in some species, the question must be raised of whether or not carbaryl reaches the fetus rather than questioning only the routes of metabolism. In addition, it must be kept in mind that any of the products of carbaryl

containing the carbamate portion of the molecule have some anti-cholinesterase properties, albeit less than carbaryl, and are capable of carbamoylating proteins (22). In these cases, it would also be important to know the concentrations of these metabolites reaching the fetuses. Although the inhibition of cholinesterase is used as an indicator of the presence of carbaryl, it is not a specific indicator. If the mode of action of carbaryl in the fetus is by cholinesterase inhibition, then it would not matter whether the inhibition resulted from carbaryl or a metabolite. However, since there are other possible actions of carbaryl, it is necessary to confirm the identity of the carbaryl in the fetus.

In the studies reported here, upon administration of radiolabeled carbaryl to the mouse or rat, radioactivity was detected in all the tissues at all time periods. Carbaryl was identified in the liver and kidney at 24 hours; in the lung and fetus at 12 hours; in the heart, eye and placenta at 8 hours; in the blood, brain and muscle at 4 hours; the skin at 2 hours; and the fat at one hour. Carbaryl could be present at later times but the sensitivity of the method precluded detection. The detection of carbaryl should have been the same regardless of the label, however there was some variation in the detection of carbaryl among the labels. The most notable was the lack of detection of carbaryl from the methyl labeled carbaryl. In contrast, a difference in the detection of metabolites from the three radiolabels was to be expected due to the presence or absence of the radiolabel in the metabolites. Since the carbon molecule from the carbonyl moiety is exhaled in the form of CO_2, it was expected to have fewer metabolites with this label, but in some tissues, there were many more metabolites with the carbonyl label than the other two labels.

Both the mouse and rat fetuses and placentas had detectable carbaryl. While very few metabolites were identified in the first study in rats, in the second study which was done at one and four hours in mice, carbaryl and a few metabolites were detected in the fetuses and placentas. In both the mouse and the rat study, the total radioactivity assayed was greater than that accounted for by carbaryl and its metabolites on the thin layer chromatograms. It is possible that the methyl- and carbonyl-radiolabel moieties were incorporated into the fetus or were bound in a carbamoyl-protein product which would produce an erroneously high estimate of carbaryl in the fetus based on total radioactivity.

In the series of studies by Declume et al. (17-20), the ^{14}C-methyl label of carbaryl was used, and an increasing concentration of label was assayed in the fetuses over 96 hours. The presence of carbaryl was not confirmed in these studies, and the increasing concentration of the radiolabel may be reflecting the incorporation of the ^{14}C-methyl group into the fetus. In an additional study, they determined the inhibition of acetylcholinesterase in the fetuses after oral administration of carbaryl on the 18th day of gestation (23). At the lowest dose level of 6.25 mg/kg, both maternal blood and brain activities were depressed at one hour. Fetal blood cholinesterase but not the fetal liver or brain activity was depressed. However, a dose of 50 mg/kg produced a significant decrease in fetal liver and brain activity in one hour. At this dose, fetal blood and brain cholinesterase activities were depressed for 12 hours while the activity in the liver was depressed for 5 hours. It is not known whether carbaryl or some of the active metabolites depressed the cholinesterease activity, but it is significant that the fetus responded with cholinesterase inhibition. However, the fetuses in these

studies were past the major stage of organogenesis so it is still not known if inhibition of cholinesterase had any bearing on a potential teratogenic action of carbaryl. In additional studies by these authors (24,25), rats were treated with low doses of 1 or 5 mg/kg of carbaryl from the 11th day to the end of gestation and were allowed to deliver their young. There was no effect on cholinesterase activity in the neonates. A higher dose of 50 mg/kg of carbaryl administered on the 19th day of gestation to birth increased the blood cholinesterase and decreased the brain and liver cholinesterase in the neonates and the birth weight. The changes in the blood and brain cholinesterase activities were seen at 14 hours after birth but not at 5 hours while the liver effects were seen at 5, 14 and 24 hours. It is not known whether these effects were from transplacental exposure of the fetus or translactational exposure.

Carbaryl and metabolites of carbaryl are excreted in the milk of cows. Carbaryl at a level of 17 ppb was confirmed in the milk of cows treated for 14 days at a dose level of 100 ppm in the diet. Three metabolites with carbamate in the molecule were also detected at levels ranging from 3 to 94 ppm (26). In three other studies, only metabolites were sought and found in the milk (27-29). It is difficult to know whether the cholinesterase inhibition in the fetuses and neonates in the rat study was due to carbaryl or metabolites of carbaryl and whether the exposure to the compounds was prenatal or postnatal or a combination of the two. This would also apply to the three-generation studies in which the number of neonates surviving to day 4 was significantly decreased.

In summary, in studies cited earlier, cholinesterase can be inhibited in the fetus with carbaryl at maternal dose levels of 50 mg/kg. Malforma-

tions can be produced in the fetus at maternal dose levels ranging from 25 to 300 mg/kg depending on the species. Reproduction can be adversely affected in the rat with chronic exposure to doses of 100-200 mg/kg. In order to establish a basis for the extrapolation of these data to the human being, the concentration of carbaryl in all the species used in the teratology studies should be determined and correlated with the teratogenic or other adverse response in reproduction thus establishing whether these differences in responses are due to species and not dose. In the current studies, carbaryl as well as some metabolites were shown to be present in the fetus by using carbaryl labeled with ^{14}C in either the naphthyl, methyl or carbonyl portion of the molecule. The naphthyl label is the best estimator of carbaryl concentration. Use of the methyl or carbonyl label may result in erroneously high estimates of carbaryl. However, it must be kept in mind that some metabolites of carbaryl could contain the active portion of the molecule, in terms of reproduction and teratology, and the resultant species difference could be related to the concentration of metabolite and/or receptor as well as the parent carbaryl.

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FIGURE LEGENDS

1. Structure of Cabaryl. Asterisks indicate the ^{14}C-naphthyl, ^{14}C-methyl and ^{14}C-carbonyl-radiolabels.

2. Distribution of ^{14}C-naphthyl, ^{14}C-methyl and ^{14}C-carbonyl labeled carbaryl equivalents in tissues of pregnant CD-1 mice at 1, 3, 5, 7 and 24 hours after an IG dose of 100 mg/kg radiolabeled carbaryl on day 13 of gestation.

3. Distribution of ^{14}C-naphthyl, ^{14}C-methyl and ^{14}C-carbonyl-labeled carbaryl equivalents in tissues of pregnant CD-1 mice at 1, 3, 5, 7 and 24 hours after an IG dose of 100 mg/kg radiolabeled carbaryl on day 13 of gestation.

4. Distribution of ^{14}C-naphthyl, ^{14}C-methyl and ^{14}C-carbonyl labeled carbaryl equivalents in tissues of pregnant CD rats at 1, 2, 4, 8, 12 and 24 hours after an IG dose of 100 mg/kg of radiolabeled carbaryl on the 14th day of gestation.

5. Distribution of ^{14}C-naphthyl, ^{14}C-methyl and ^{14}C-carbonyl labeled carbaryl equivalents in tissues of pregnant CD rats at 1, 2, 4, 8, 12 and 24 hours after an IG dose of 100 mg/kg of radiolabeled carbaryl on the 14th day of gestation.

6. Excretion of the radiolabeled carbaryl equivalents in the pooled urine and pooled feces of the CD-1 mouse and CD rat of study one

24 hours after a single IG dose of 100 mg/kg expressed as mg excreted in 24 hours.

7. Excretion of the radiolabeled carbaryl equivalents in the pooled urine and pooled feces of the CD-1 mouse and CD rat of study one 24 hours after a single IG dose of 100 mg/kg per animal expressed as percent of dose excreted.

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Table 1. Study 1. Fetal and Placental Concentrations of ^{14}-C-Naphthyl, ^{14}-C-methyl, ^{14}-C-Carbonyl Carbaryl Equivalents in Mice and Rats.[a]

Hr.	Number Mice (Assays)[b]	Mouse Fetus µg/g	Mouse Placenta µg/g	Hr.	Number Rats (Assays)	Rat Fetus µg/g	Rat Placenta µg/g
		^{14}C-NAPHTHYL - CARBARYL				^{14}C-NAPHTHYL - CARBARYL	
1	1 (7)	2.4	7.1	1	2 (6)	4.6 ± 0.9	9.3 ± 1.6
3	2 (10)	1.9 ± 0.6	8.2 ± 2.7	2	2 (4)	3.9 ± 1.4	8.1 ± 3.1
5	2 (11)	2.0 ± 0.1	7.0 ± 1.7	4	2 (4)	2.8 ± 1.0	3.1 ± 0.9
7	1 (6)	1.9	5.6	8	2 (4)	2.2 ± 0.2	5.5 ± 0.5
24	2 (15)	0.2 ± 0.0	1.4 ± 0.1	12	2 (4)	1.7 ± 0.3	4.5 ± 0.0
				24	3 (6)	1.8 ± 0.8	1.8 ± 0.7
		^{14}C-METHYL - CARBARYL				^{14}C-METHYL - CARBARYL	
1	1 (4)	12.6	23.1	1	2 (4)	7.4 ± 2.8	11.2 ± 4.7
3	2 (10)	19.7 ± 0.5	24.6 ± 1.9	2	2 (4)	5.1 ± 1.1	8.8 ± 2.3
5	1 (7)	23.5	28.5	4	2 (4)	9.1 ± 3.3	12.4 ± 4.1
7	2 (8)	23.2 ± 0.3	25.1 ± 1.4	8	2 (4)	3.9 ± 0.1	8.8 ± 2.1
24	1 (4)	10.1	12.3	12	2 (4)	11.7 ± 1.9	16.4 ± 1.2
				24	1 (3)	15.0	23.5
		^{14}C-CARBONYL - CARBARYL				^{14}C-CARBONYL - CARBARYL	
1	2 (2)	3.0 ± 0.3	7.3 ± 1.4	1	2 (4)	6.0 ± 0.6	10.4 ± 1.0
3	2 (10)	4.3 ± 0.6	6.9 ± 0.2	2	2 (5)	4.7 ± 3.4	7.5 ± 5.4
5	2 (10)	5.8 ± 0.7	8.0 ± 1.3	4	2 (4)	5.8 ± 0.8	8.9 ± 0.9
7	2 (10)	8.0 ± 0.4	10.2 ± 1.8	8	2 (4)	6.2 ± 2.0	10.1 ± 3.8
24	1 (5)	2.8	5.0	12	2 (4)	4.9 ± 0.6	7.7 ± 0.2
				24	2 (4)	5.9 ± 0.6	8.1 ± 0.8

[a]Data presented on a per litter (per mouse) basis.

[b]Number of pools of fetuses assayed; one to three fetuses per pool.

Table 2. Study one. Rat Tissue Assayed for Presence of Carbaryl by Thin-Layer Chromatography. "C" Indicates Carbaryl was Detected. Metabolites are Indicated by Numbers which are the Metabolite's Rf as a Percent of Carbaryl Rf. Dash indicates nothing detected.

TISSUE	LABEL	1 hr.	2 hr.	4 hr.	8 hr.	12 hr.	24 hr.
LIVER	NAPH	C	C	-	C	C	-
		31	16	-	10	16	-
		-	13	-	-	-	-
	METH	-	C	-	-	-	C
	CARB	C	C	C	-	C	C
		148	139	120	116	126	143
		124	51	-	111	-	132
		24	9	-	14	-	124
		-	-	-	-	-	78
		-	-	-	-	-	66
		-	-	-	-	-	50
		-	-	-	-	-	40
		-	-	-	-	-	25
		-	-	-	-	-	20
		-	-	-	-	-	9
KIDNEY	NAPH	C	-	C	C	-	-
		88	17	79	113	117	-
		80	-	-	11	62	-
		65	-	-	-	52	-
		18	-	-	-	40	-
		1	-	-	-	34	-
		-	-	-	-	23	-
		-	-	-	-	13	-
		-	-	-	-	10	-
	METH	-	C	-	-	C?	C
		-	-	-	-	139	-
		-	-	-	-	29	-
		-	-	-	-	11	-
		-	-	-	-	7	-
		-	-	-	-	4	-
	CARB	C	-	C	C	C	C
		24	-	125	9	73	148
		-	-	11	-	63	-
HEART	NAPH	C	C	C	C	-	-
		-	15	-	-	-	-
	METH	-	-	-	-	-	-
	CARB	C	-	C	C	-	-
		-	-	124	123	130	161
		-	-	118	9	8	150
		-	-	92	-	-	81
		-	-	7	-	-	-

Table 2 continued.

TISSUE	LABEL	1 hr.	2 hr.	4 hr.	8 hr.	12 hr.	24 hr.
LUNG	NAPH	C	C	-	-	C	-
		-	-	-	-	13	-
	METH	-	C	-	-	-	-
	CARB	C	-	C	-	-	-
		114	-	129	129	130	126
		24	-	-	122	-	115
		-	-	-	-	-	58
		-	-	-	-	-	46
SKIN	NAPH	C	C	-	-	-	-
		138	-	-	10	-	-
		18	-	-	-	-	-
	METH	C?	C?	C?	C?	-	-
	CARB	C	-	C	-	-	-
		-	-	2	-	-	-
BRAIN	NAPH	C	-	-	-	-	-
		15	63	-	-	-	-
		-	13	-	-	-	-
	METH	C?	C?	-	-	-	C?
	CARB	C	-	C	C?	-	-
		127	-	127	119	163	123
		-	-	121	8	-	118
FAT	NAPH	C?	C?	-	-	-	-
	METH	-	C?	-	C?	-	C?
	CARB	C	-	C?	-	-	-
		71	-	67	-	-	-
MUSCLE	NAPH	C	C	-	-	-	-
		-	-	-	8	44	-
		-	-	-	-	38	-
		-	-	-	-	16	-
		-	-	-	-	11	-
	METH	-	C?	-	-	-	-
	CARB	C	-	C	-	-	-
		173	-	-	128	2	17

Table 2 continued.

TISSUE	LABEL	1 hr.	2 hr.	4 hr.	8 hr.	12 hr.	24 hr.
EYE	NAPH	-	C	-	-	-	-
	METH	-	-	-	-	-	-
	CARB	C?	-	C	C	-	C?
		134	-	-	-	-	-
		32	-	-	-	-	-
		12	-	-	-	-	-
BLOOD	NAPH	-	C	-	-	-	-
	METH	-	-	-	-	-	-
	CARB	C	C	C	-	-	-
		-	5	-	-	-	-
FETUS	NAPH	C	C	-	C	C	-
		112	-	-	-	10	-
	METH	-	-	-	C?	-	-
	CARB	-	-	-	C?	-	-
		-	-	-	-	-	66
PLACENTA	NAPH	C	C	-	C	-	-
		25	14	-	-	-	-
	METH	-	-	-	-	-	-
	CARB	C	C	C	C	-	-
		-	-	-	11	-	-

Table 3. Study two. Mouse Tissue Concentrations of ^{14}-C-Naphthyl, ^{14}-C-Methyl or ^{14}-C-Carbonyl Carbaryl Equivalents One or Four Hours After Treatment.[a]

Tissue	Hr.	Sample	^{14}-C-Carbaryl Naphthyl µg/g	^{14}-C-Carbaryl Methyl µg/g	^{14}-C-Carbaryl Carbonyl µg/g
Liver	1	Whole	59.7 ± 18.2	30.9 ± 8.3[b]	10.0 ± 3.7[b]
	4	Whole	34.9 ± 3.5	53.7 ± 3.1[b]	41.6 ± 7.2[b]
	1	Homog	57.5 ± 15.0	37.2 ± 11.8	9.7 ± 3.3[b]
	4	Homog	54.8 ± 10.9	56.0 ± 7.3	54.7 ± 8.3[b]
Kidney	1	Whole	49.4 ± 9.3	24.7 ± 6.7[b]	8.7 ± 3.3[b]
	4	Whole	44.7 ± 6.1	46.2 ± 5.8[b]	39.4 ± 9.5[b]
	1	Homog	38.1 ± 4.2	19.7 ± 6.1[b]	9.8 ± 3.1[b]
	4	Homog	54.2 ± 15.9	44.6 ± 3.3[b]	43.6 ± 13.6[b]
Lung	1	Whole	23.0 ± 5.1	5.1 ± 1.5[b]	2.3 ± 0.7[b]
	4	Whole	12.7 ± 1.9	18.2 ± 2.9[b]	17.8 ± 5.2[b]
	1	Homog	26.2 ± 2.0[b]	11.8 ± 3.9	2.5 ± 1.4[b]
	4	Homog	9.4 ± 0.2[b]	14.3 ± 0.9	26.4 ± 4.4[b]
Skin	1	Whole	24.0 ± 11.2	52.2 ± 18.0	2.7 ± 1.1
	4	Whole	10.3 ± 1.7	12.9 ± 0.9[b]	9.7 ± 3.1[b]
	1	Homog	30.7 ± 16.9	12.8 ± 6.2	4.4 ± 1.4
	4	Homog	8.5 ± 1.7	9.4 ± 1.3	10.3 ± 3.9
Muscle	1	Whole	10.3 ± 3.7	4.3 ± 1.0	1.0 ± 0.4
	4	Whole	10.9 ± 6.0	11.3 ± 2.4	5.5 ± 1.2
	1	Homog	11.1 ± 4.7	6.2 ± 2.0	2.1 ± 1.2
	4	Homog	8.9 ± 3.5	13.1 ± 4.7	8.1 ± 2.9
Fat	1	Whole	9.8 ± 2.6	12.8 ± 7.7	2.4 ± 1.3
	4	Whole	9.8 ± 3.4	10.0 ± 1.9	6.4 ± 2.5
	1	Homog	8.2 ± 1.8	5.9 ± 2.6	3.6 ± 2.3
	4	Homog	9.2 ± 3.9	7.9 ± 2.3	16.1 ± 9.0
Blood	1	Whole	14.6 ± 2.0	8.2 ± 2.0[b]	3.0 ± 1.1[b]
	4	Whole	11.2 ± 1.6	19.5 ± 2.5[b]	18.2 ± 3.7[b]
Uterus	1	Whole	10.0 ± 1.7	6.8 ± 1.7[b]	2.0 ± 0.6[b]
	4	Whole	9.7 ± 1.3	11.8 ± 0.7[b]	11.0 ± 3.1[b]
	1	Homog	11.1 ± 2.4	8.0 ± 1.7	1.6 ± 0.4[b]
	4	Homog	11.8 ± 2.6	12.7 ± 1.7	17.0 ± 6.4[b]

Table 3 continued.

Tissue	Hr.	Sample	^{14}-C-Carbaryl Naphthyl µg/g	Methyl µg/g	Carbonyl µg/g
Eye	1	Whole	2.3 ± 0.4	5.4 ± 1.7	0.4 ± 0.1[b]
	4	Whole	2.3 ± 0.3	9.4 ± 2.2	4.9 ± 1.4[b]
	1	Homog	-	8.3 ± 4.6	0.1
	4	Homog	4.2 ± 2.2	-	15.0 ± 0.8
Brain	1	Whole	1.2 ± 0.2	2.5 ± 0.8[b]	0.3 ± 0.1[b]
	4	Whole	1.0 ± 0.1	4.9 ± 0.3[b]	6.4 ± 2.0[b]
	1	Homog	1.7 ± 0.3	5.7 ± 1.5[b]	0.6 ± 0.2[b]
	4	Homog	1.3 ± 0.3	21.6 ± 6.9[b]	6.2 ± 1.6[b]

[a]Means ± SE of 5 or 6 mice at each time period for each radiolabel.

[b]$p = < 0.05$ comparing 4 hour values to their respective 1 hour values.

Table 4. Study two. Rat Tissue Concentrations of ^{14}-C-Naphthyl or ^{14}-C-Methyl Carbaryl Equivalents One or Four Hours After Treatment.[a]

Tissue	Hr.	^{14}-C-Naphthyl µg/g	^{14}-C-Methyl µg/g
Liver	1	28.4 ± 4.5	49.1 ± 5.5
	4	21.7 ± 2.0	47.8 ± 3.1
Kidney	1	29.8 ± 4.5	48.7 ± 8.8
	4	29.3 ± 1.6	45.8 ± 2.7
Lung	1	13.8 ± 2.8	24.0 ± 4.9
	4	12.9 ± 2.4	18.4 ± 2.5
Skin	1	8.3 ± 1.5	21.5 ± 10.6
	4	3.8 ± 0.5[b]	7.7 ± 0.7
Fat	1	14.0 ± 3.0[b]	16.9 ± 2.5
	4	7.3 ± 1.6	7.4 ± 1.7[b]
Muscle	1	6.5 ± 1.1	9.4 ± 1.5
	4	6.8 ± 2.2	11.1 ± 1.1
Brain	1	7.0 ± 1.8	11.0 ± 2.1
	4	2.6 ± 0.8	13.1 ± 0.9
Heart	1	9.6 ± 2.0	12.9 ± 1.0
	4	6.5 ± 0.9	15.3 ± 1.8
Eye	1	6.0 ± 2.8	6.6 ± 1.5
	4	2.2 ± 0.2	5.2 ± 0.8
Blood	1	14.9 ± 3.1	15.9 ± 1.7
	4	8.4 ± 0.8	21.1 ± 1.4[b]
Serum	1	31.5 ± 7.2	18.8 ± 2.5
	4	- -	47.1 ± 2.9[b]

[a]Means ± SE of 5 or 6 rats at each time period for each radiolabel.

[b]$p = < 0.05$ comparing 4 hour values to their respective 1 hour values.

Table 5. Study 2. Mouse and Rat Fetal and Placental Concentrations of Radiolabeled Carbaryl Equivalents One or Four Hours After Treatment.[a]

Hr.	Number Mice (Assays)	Tissue	Mouse Fetus µg/g	Mouse Placenta µg/g
			^{14}-C-Naphthyl - Carbaryl	
1	6 (11)	Whole	1.76 ± 0.32	5.64 ± 0.87
4	5 (10)	Whole	2.16 ± 0.23	6.44 ± 0.61
1	6 (12)	Homog	1.84 ± 0.23	5.83 ± 0.56
4	5 (10)	Homog	3.08 ± 0.48[b]	7.13 ± 0.89
			^{14}-C-Methyl - Carbaryl	
1	5 (10)	Whole	2.88 ± 0.78	3.21 ± 0.64
4	5 (10)	Whole	10.10 ± 1.49[b]	16.97 ± 1.44[b]
1	4 (8)	Homog	3.26 ± 0.63	3.71 ± 1.07
4	5 (10)	Homog	13.96 ± 1.80[b]	22.13 ± 3.25[b]
			^{14}-C-Carbonyl - Carbaryl	
1	6 (11)	Whole	0.42 ± 0.21	0.94 ± 0.31
4	4 (8)	Whole	7.39 ± 1.50[b]	9.53 ± 1.52[b]
1	6 (12)	Homog	0.55 ± 0.15	1.57 ± 0.44
4	4 (8)	Homog	10.00 ± 3.06[b]	9.45 ± 2.54[b]

Hr.	Number Rats (Assays)	Tissue	Rat Fetus µg/g	Rat Placenta µg/g
			^{14}-C-Naphthyl - Carbaryl	
1	6 (22)	Homog	4.59 ± 1.29	8.22 ± 1.53
4	4 (14)	Homog	2.43 ± 0.36	4.43 ± 0.48
			^{14}-C-Methyl - Carbaryl	
1	4 (16)	Homog	7.24 ± 1.67	12.03 ± 2.58
4	5 (17)	Homog	10.34 ± 1.09	14.88 ± 2.58

[a]Means ± SE.

[b]$p = < 0.05$ comparing 4 hour values to their respective 1 hour values.

Table 6. Study Two. Mouse Tissue Assayed for Presence of Carbaryl by Thin-Layer Chromatography One or Four Hours After Treatment with a Radiolabeled Carbaryl. The Rf of the Metabolites are Presented as the Percent of the Carbaryl Rf.

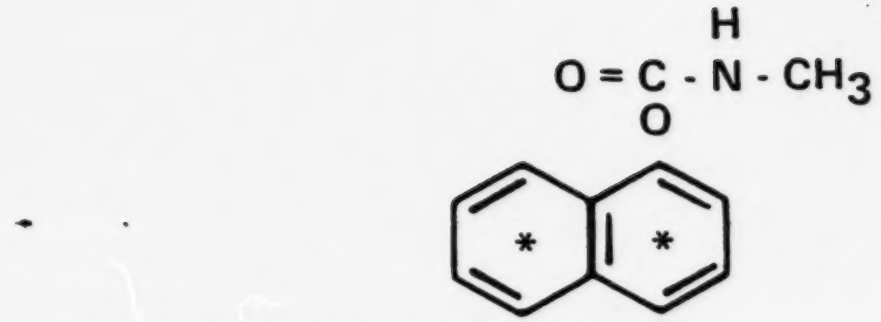
Radio-Lable	Hr	No. Mice	No. Mice With Carbaryl	No. Mice With Metabolites Rf as PERCENT OF CARBARYL RF 8-15	25-40	45-50	52-90	110-130	132-200
Liver									
Naphthyl	1	5	1	3	-	3	-	4	4
Naphthyl	4	5	3	-	1	3	-	3	-
Methyl	1	4	-	-	-	-	-	1	2
Methyl	4	6	1	-	1	-	-	1	5
Carbonyl	1	5	4	-	-	1	-	4	3
Carbonyl	4	4	3	1	-	-	-	1	1
Kidney									
Naphthyl	1	5	3	1	2	4	-	4	4
Naphthyl	4	5	1	-	1	4	-	-	-
Methyl	1	4	2	1	-	-	-	3	3
Methyl	4	5	-	-	2	1	-	-	5
Carbonyl	1	7	1	-	-	1	-	1	3
Carbonyl	4	7	3	1	-	-	-	-	4
Placenta									
Naphthyl	1	6	4	-	-	1	2	-	-
Naphthyl	4	5	-	-	-	2	2	-	-
Methyl	1	6	2	-	-	-	-	1	-
Methyl	4	6	1	-	-	-	-	-	-
Carbonyl	1	6	-	-	-	-	-	-	1
Carbonyl	4	4	-	-	-	-	-	2	-

Fetus									
Naphthyl	1	6	3	-	-	-	-	-	-
Methyl	1	6	1	-	-	-	2	1	-
Carbonyl	1	6	-	-	-	-	1	2	-
Carbonyl	4	4	2	-	-	-	-	2	-

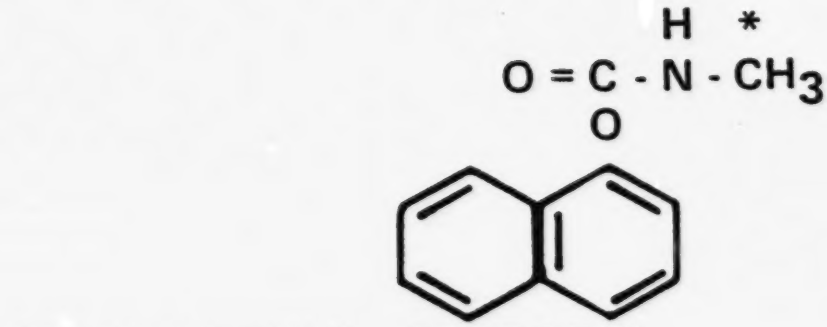
Table 7. Study Two. Rat Tissue Assayed for Presence of Carbaryl by Thin-layer Chromatography One or Four Hours After Treatment with Either ^{14}C-Naphthyl or ^{14}-C-Methyl Carbonyl. The Rf of the Metabolites are Presented as the Percent of the Carbaryl Rf.

Tissue	No. Rats With Carbaryl	No. Rats With Metabolites Rf as PERCENT OF CARBARYL Rf 25-40	52-90	110-130	132-200	No. Rats With Carbaryl	No. Rats With Metabolites Rf as PERCENT OF CARBARYL Rf 25-40	52-90	110-130	132-200
	^{14}C-naphthyl-One Hour[a]					^{14}C-Methyl-One Hour[b]				
Liver	1	-	-	1	-	1	-	-	-	-
Kidney	3	-	-	3	-	2	-	1	1	1
Lung	5	-	-	1	1	-	-	1	1	2
Skin	2	-	-	-	-	1	-	-	1	1
Fat	3	-	-	2	2	2	-	-	-	-
Muscle	2	-	-	1	-	4	-	-	-	1
Brain	1	-	-	1	-	-	-	2	-	-
Heart	4	-	-	-	-	2	-	-	2	-
Eyes	2	-	-	1	-	1	-	-	1	-
Fetus	2	-	-	2	-	2	-	-	1	-
Placenta	2	-	-	1	-	3	-	1	-	1
	^{14}-C-Naphthyl - Four Hour[c]					^{14}-C-Methyl - Four Hour[d]				
Liver	1	2	-	1	-	-	-	-	-	-
Kidney	-	-	-	3	1	-	-	-	1	1
Lung	-	-	-	5	-	1	-	-	2	-
Skin	-	-	-	-	2	2	-	-	1	-
Fat	-	-	-	1	1	1	-	-	-	1
Muscle	3	-	-	-	-	-	-	-	1	-
Brain	-	-	-	3	-	1	-	-	-	2
Heart	1	-	-	2	1	-	-	-	1	-
Eyes	1	-	-	1	-	-	-	-	2	-
Fetus	-	-	-	1	-	-	-	-	-	-
Placenta	2	-	-	3	-	3	-	-	1	-

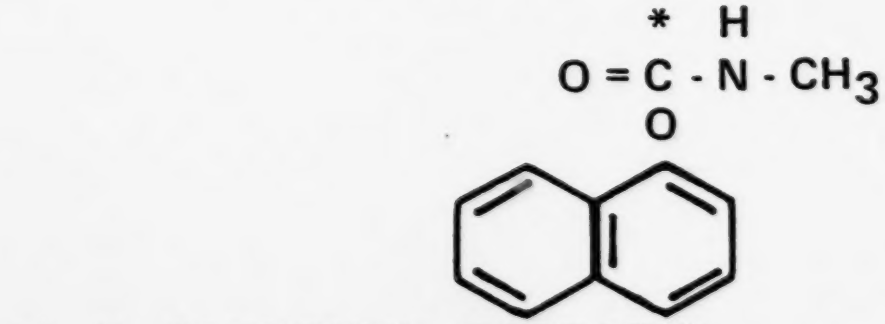
a: 6 Rats; b: 5 Rats; c: 4 Rats; d: 5 Rats



14 - C - NAPHTHYL - CARBARYL



14 - C - METHYL - CARBARYL



14 - C - CARBONYL - CARBARYL

Figure 1.

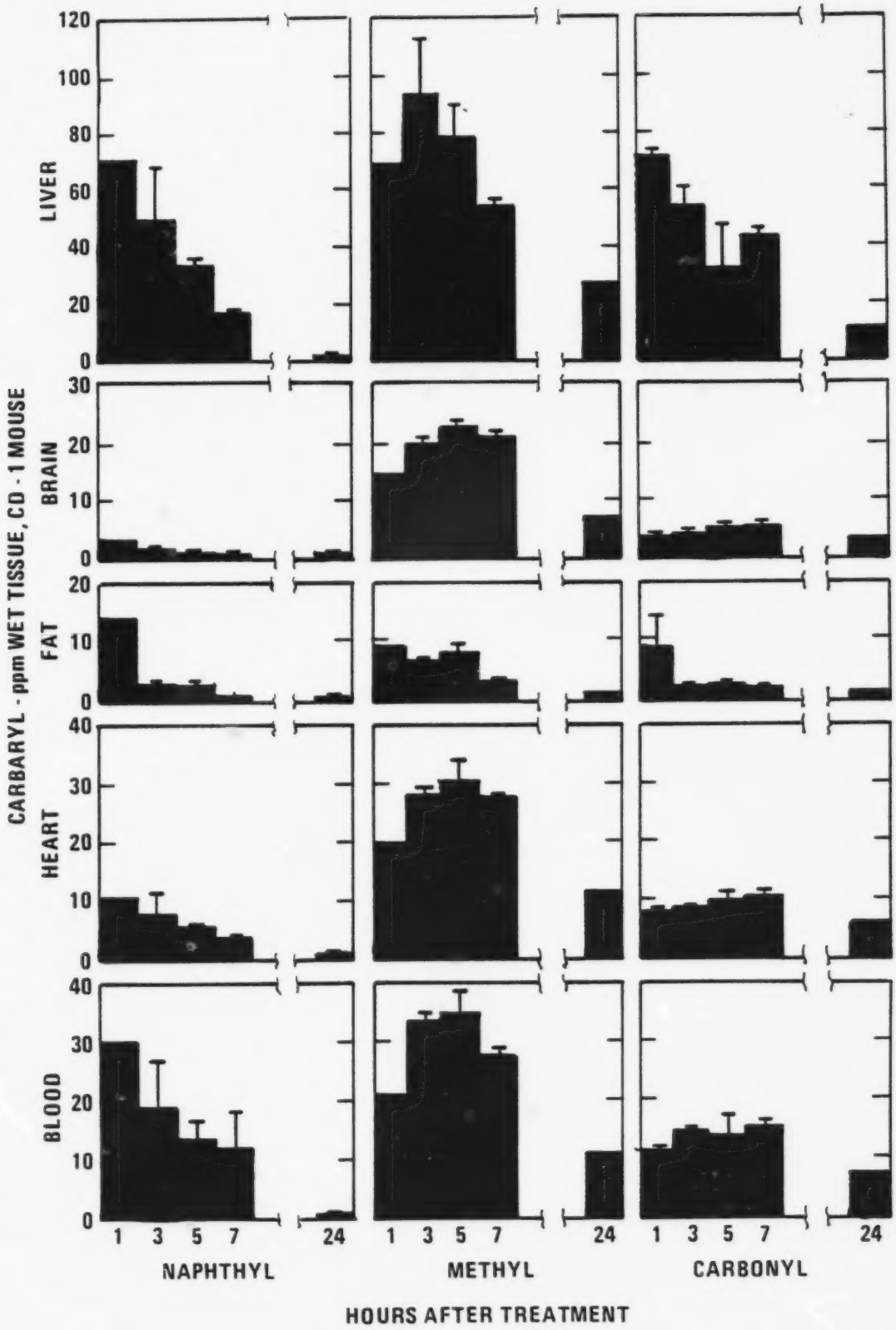

CARBARYL - ppm WET TISSUE, CD - 1 MOUSE
LIVER
BRAIN
FAT
HEART
BLOOD
120
100
80
60
40
30
20
10
0
1 3 5 7
24
NAPHTHYL
METHYL
CARBONYL
HOURS AFTER TREATMENT

Figure 2.

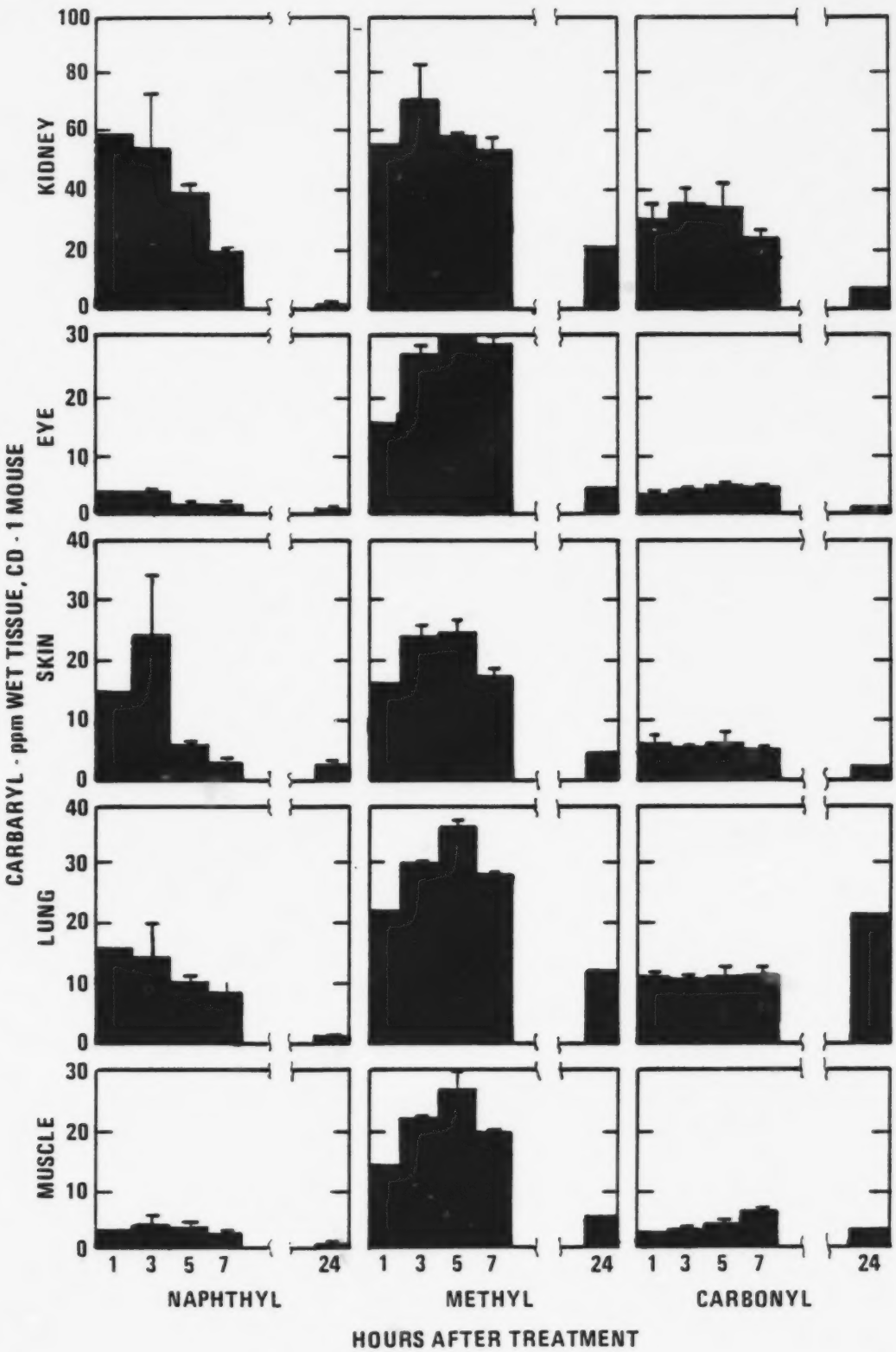

CARBARYL - ppm WET TISSUE, CD - 1 MOUSE
KIDNEY
EYE
SKIN
LUNG
MUSCLE
100
80
60
40
20
0
30
20
10
0
40
30
20
10
0
40
30
20
10
0
30
20
10
0
1 3 5 7 24
NAPHTHYL
METHYL
CARBONYL
HOURS AFTER TREATMENT

Figure 3.

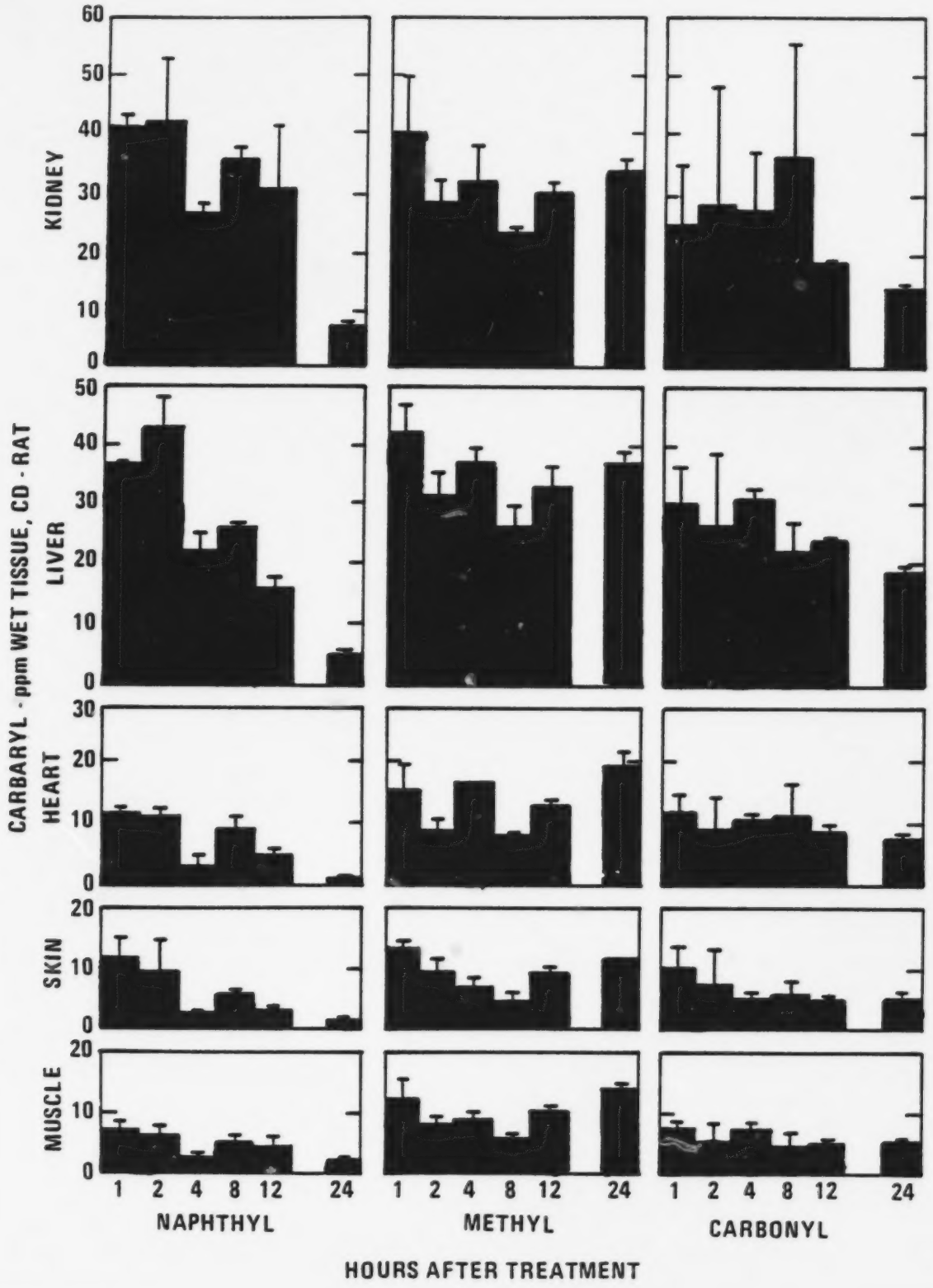

CARBARYL - ppm WET TISSUE, CD - RAT
KIDNEY
LIVER
HEART
SKIN
MUSCLE
0
10
20
30
40
50
60
1 2 4 8 12 24
NAPHTHYL
METHYL
CARBONYL
HOURS AFTER TREATMENT

Figure 4.

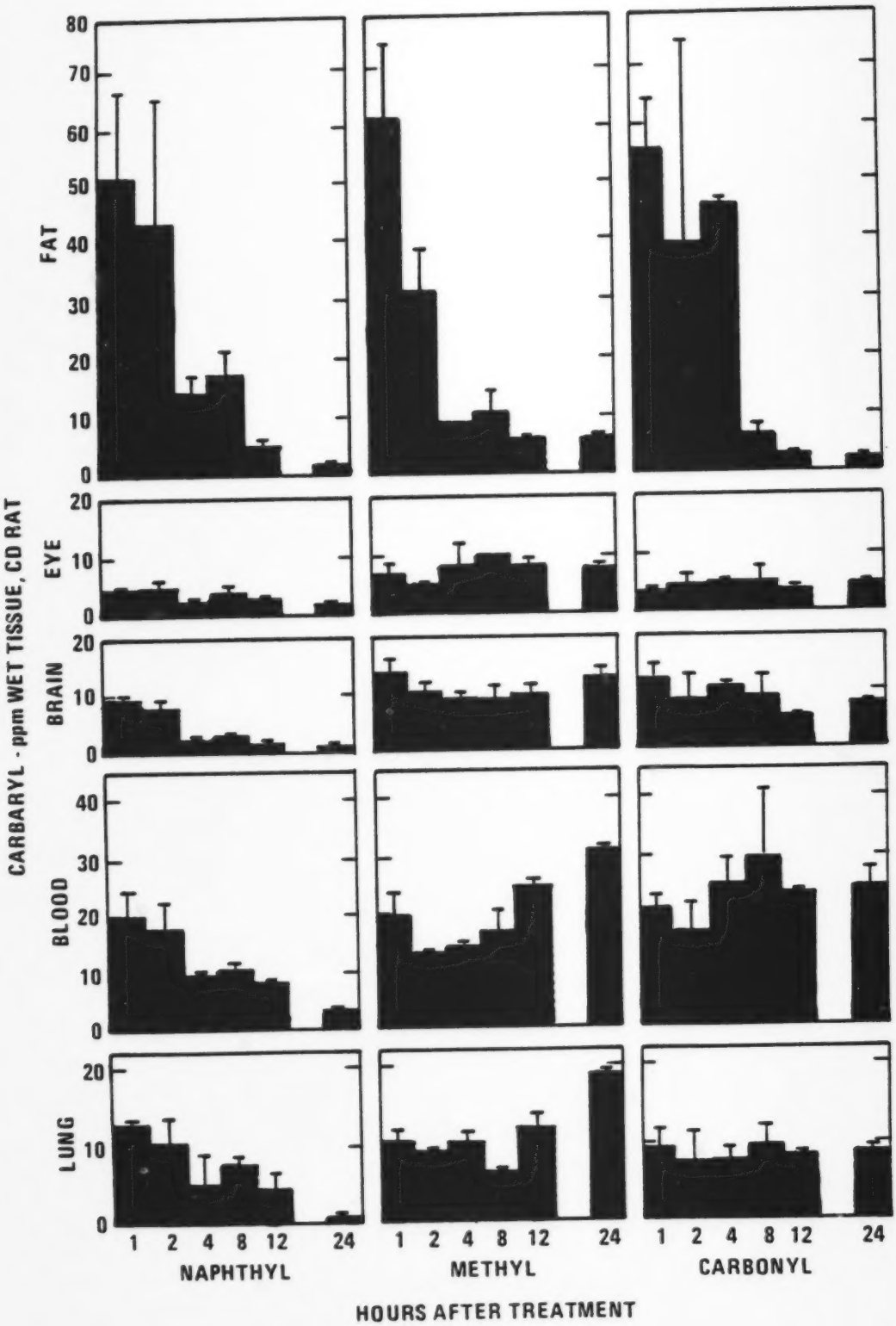

CARBARYL - ppm WET TISSUE, CD RAT
FAT
EYE
BRAIN
BLOOD
LUNG
1 2 4 8 12 24
NAPHTHYL
METHYL
CARBONYL
HOURS AFTER TREATMENT

Figure 5.

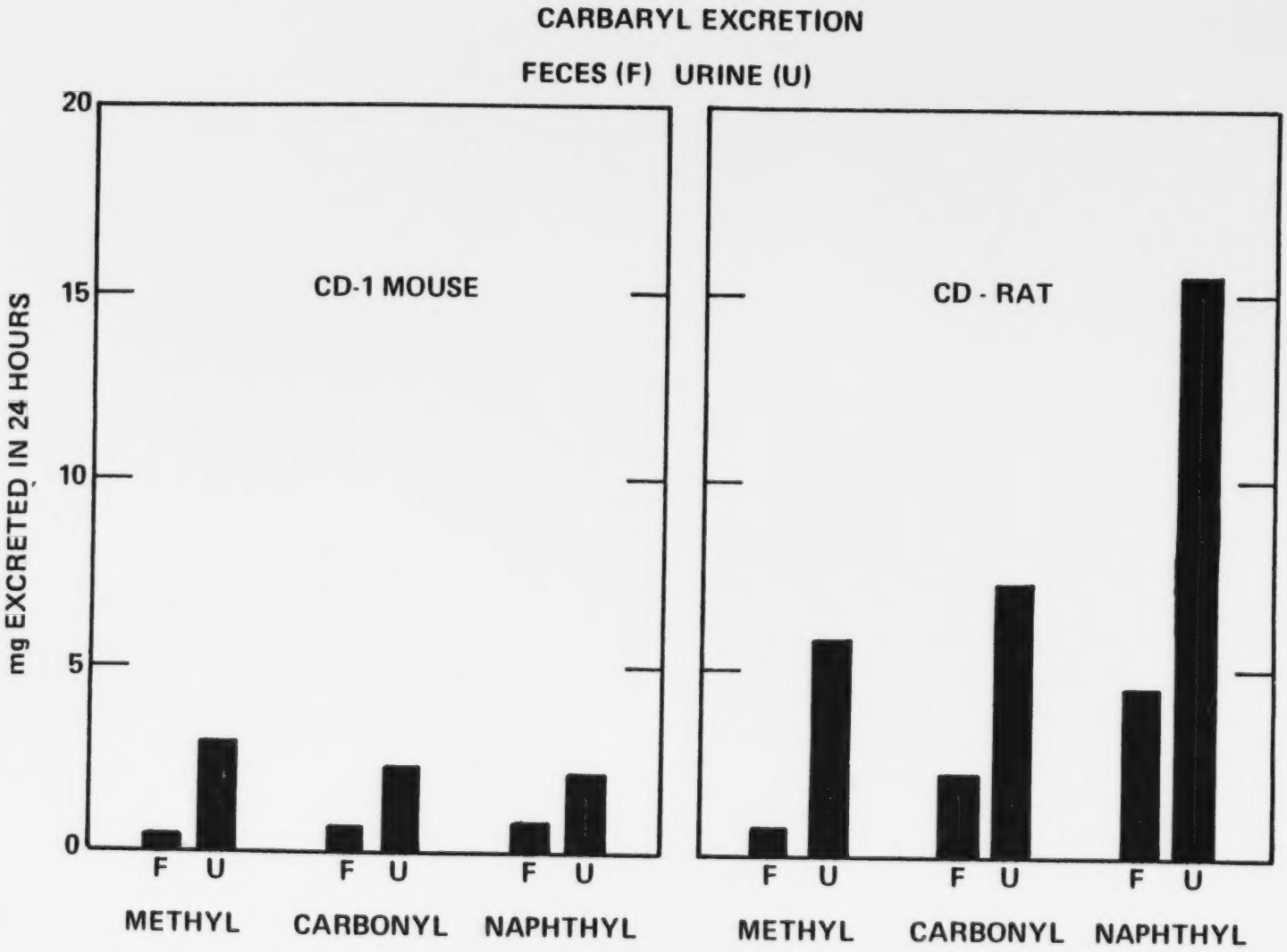

CARBARYL EXCRETION
FECES (F) URINE (U)
mg EXCRETED IN 24 HOURS
20
15
10
5
0
CD-1 MOUSE
CD - RAT
F U
METHYL
CARBONYL
NAPHTHYL

Figure 6.

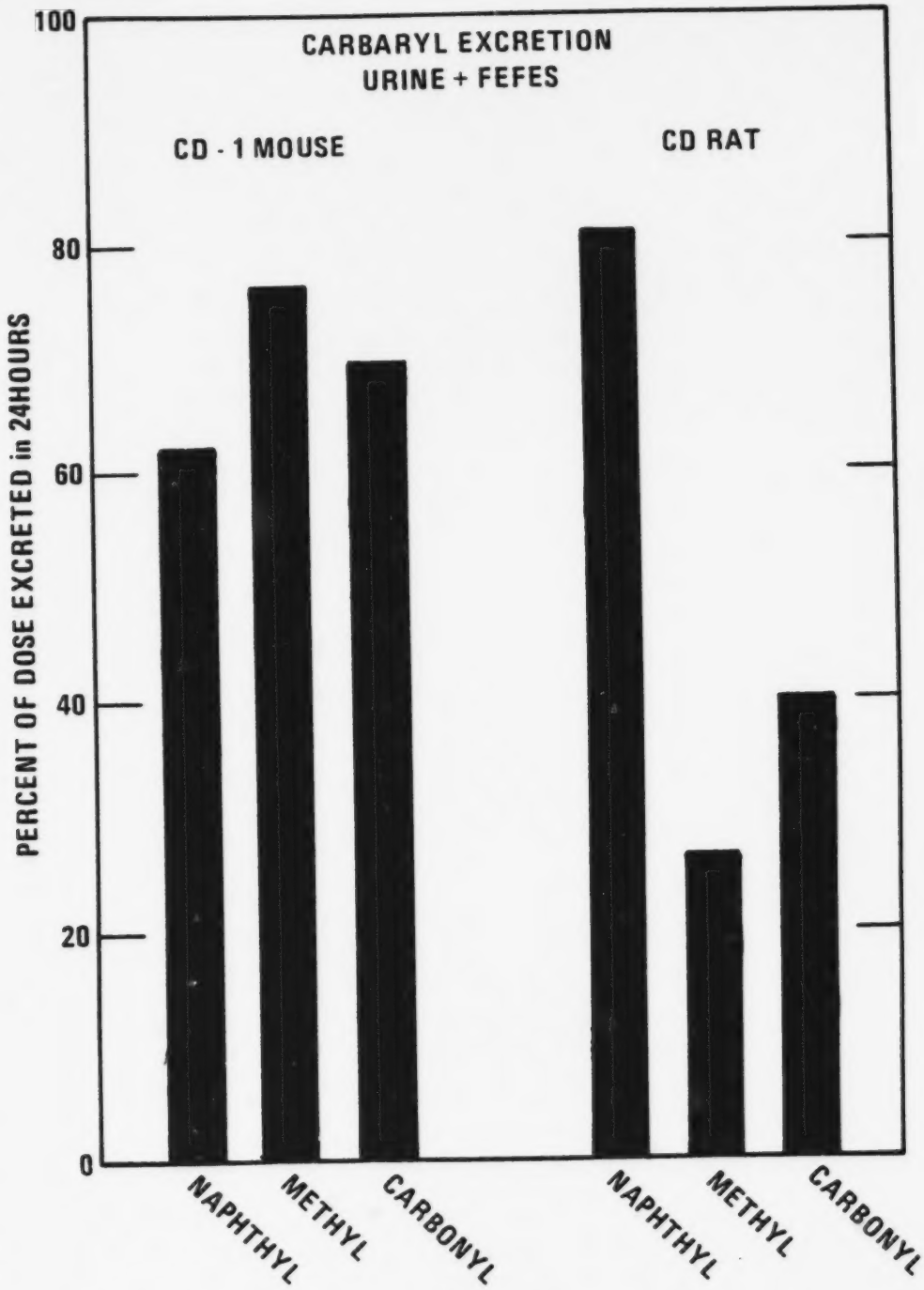

CARBARYL EXCRETION
URINE + FEFES
CD - 1 MOUSE
CD RAT
PERCENT OF DOSE EXCRETED in 24HOURS
100
80
60
40
20
0
NAPHTHYL
METHYL
CARBONYL
NAPHTHYL
METHYL
CARBONYL

Figure 7.